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(73) Proprietor: **Biosceptre (Aust) Pty Ltd**

North Ryde NSW 2113 (AU)

(72) Inventors:

- **GIDLEY-BAIRD, Angus**
North Ryde, NSW 2113 (AU)
- **BARDEN, Julian, Alexander**
North Ryde, NSW 2113 (AU)

(74) Representative: **Gregson, Anna Louise et al**

Mathys & Squire LLP
The Shard
32 London Bridge Street
London
SE1 9SG (GB)

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• **WORTHINGTON<A> R A ET AL:** "Point mutations confer loss of ATP-induced human P2X7 receptor function", **FEBS LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 512, no. 1-3, 13 February 2002 (2002-02-13), pages 43-46, XP004341347, ISSN: 0014-5793, DOI: 10.1016/S0014-5793(01)03311-7**

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Description**Field of the disclosure**

5 **[0001]** The disclosure relates to purinergic (P2X) receptors, to generation of antibodies and to use of antibodies and immunogens for detection and treatment of a disease or condition, especially cancer.

Background of the disclosure

10 **[0002]** Purinergic (P2X) receptors are ATP-gated cation -selective channels. Each receptor is made up of three protein subunits or monomers. To date seven separate genes encoding P2X monomers have been identified: P2X1, P2X2, P2X3, P2X4, P2X5, P2X6, P2X7.

[0003] P2X7 receptors are of particular interest as the expression of these receptors is understood to be limited to cells having potential to undergo programmed cell death, such as thymocytes, dendritic cells, lymphocytes, macrophages and monocytes. There is some expression of P2X7 receptors in normal homeostasis, such as on erythrocytes.

15 **[0004]** Interestingly, a P2X7 receptor containing one or more monomers having a cis isomerisation at Pro210 (according to SEQ ID NO: 1 in Figure 1) and which is devoid of ATP binding function has been found on cells that are understood to be unable to undergo programmed cell death, such as preneoplastic cells and neoplastic cells.

[0005] Antibodies generated from immunisation with a peptide including Pro210 in cis bind to P2X7 receptors that are devoid of ATP binding function. However, they do not bind to P2X7 receptors capable of binding ATP. Accordingly, these antibodies are useful for selectively detecting many forms of carcinoma and haemopoietic cancers and to treatment of some of these conditions.

20 **[0006]** The region of the P2X7 receptor containing Pro210 forms part of the extra cellular domain of the receptor. Antibodies raised against other epitopes on this domain, including those that bind regions including from Val71 to Val87 (according to SEQ ID NO: 1 in Figure 1) and from Lys 137 to Cys152 (according to SEQ ID NO:1 in Figure 1) have been found not to be capable of selectively binding to the receptor that is devoid of ATP binding function. Hence, other than the antibodies directed to the region including Pro210, no other antibodies have been found to date to be able to discriminate between ATP and non ATP binding receptors.

25 **[0007]** There is a need for reagents for detection of cancer and in this context, for new antibodies capable of discriminating between ATP and non ATP binding P2X7 receptors. There is also a need for new cancer therapeutics, including antibodies and immunogens for providing an anti tumour response.

30 **[0008]** Cheewatrakoolpong et al. (Biochem. & Biophys. Res. Comm. (2005) 332(1): 17-27) describes the identification and characterization of splice variants of the human P2X7 ATP channel. UniProt Accession Nos. Q4VK11_Human and Q4VK10_Human are human P2X7 receptor sequences. Barden et al. (FEBS Letters (2003) 538: 159-162) describes the specific detection of non-functional human P2X7 receptors in HEK293 cells and B-lymphocytes. Worthington et al. (FEBS Letters (2002) 512: 43-46) describes point mutations which confer the loss of ATP-induced human P2X7 receptor function. WO 02/057306 describes antibodies to non-functional P2X7 receptors and diagnosis and treatment of cancers and other conditions. WO 2007/027957 describes reagents for the detection of protein phosphorylation in leukaemia signalling pathways

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Summary of the disclosure

[0009] The invention is defined by the claims. Those aspects/instances of the present disclosure which constitute the present invention are defined by the claims.

45 **[0010]** In certain instances there is provided a peptide:

- consisting of the sequence of SEQ ID NO: 2;
- consisting of a sequence within the sequence of SEQ ID NO: 2, said peptide being useful as an immunogen to generate an antibody that is capable of selectively binding to a non ATP -binding P2X7 receptor but not to an ATP -binding P2X7 receptor;
- consisting of a sequence of SEQ ID NO: 3, said peptide being useful as an immunogen to generate an antibody that is capable of selectively binding to a non ATP -binding P2X7 receptor but not to an ATP -binding P2X7 receptor; or
- consisting of a sequence within the sequence of SED ID NO: 3, said peptide being useful as an immunogen to generate an antibody that is capable of selectively binding to a non ATP -binding P2X7 receptor but not to an ATP -binding P2X7 receptor.

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[0011] In other instances there is provided an antibody or fragment thereof:

- capable of binding to a peptide described above; or
- capable of binding to an epitope that includes one or more residues of a peptide having a sequence of SEQ ID NO: 2.

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[0012] In other instances there is provided an immune complex formed from the binding of an antibody or fragment thereof described above to a non ATP -binding P2X7 receptor, monomer or fragment thereof, or to a peptide as described above.

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[0013] In certain instances there is provided a method for determining whether a cell, tissue or extra cellular body fluid includes a non ATP -binding P2X7 receptor, monomer or fragment thereof including:

- contacting a cell, tissue or extra cellular body fluid with an antibody or fragment thereof in conditions for forming an immune complex as described above, and

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- detecting whether an immune complex has been formed,

wherein detection of an immune complex determines that a cell, tissue or extra -cellular body fluid includes a non ATP -binding P2X7 receptor, monomer or fragment thereof.

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[0014] In other instances there is provided a method for determining whether a cell, tissue or extra -cellular body fluid contains an antibody against a non ATP -binding P2X7 receptor, monomer or fragment thereof including:

- contacting a cell, a tissue or an extra -cellular body fluid with a peptide as described above in conditions for forming an immune complex between the peptide and an antibody in the cell, tissue or extra -cellular body fluid, and

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- detecting whether an immune complex has been formed,

wherein detection of an immune complex determines that a cell, tissue or extra -cellular body fluid contains an antibody against a non ATP -binding P2X7 receptor, monomer or fragment thereof.

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[0015] In yet further instances there is provided a kit or composition for determining whether a cell, tissue or extra -cellular body fluid contains a non ATP -binding P2X7 receptor, monomer or fragment thereof, or an antibody against same including:

- a peptide as described above; and/or

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- an antibody or fragment thereof as described above; and/or

- non ATP -binding P2X7 receptor, monomer or fragment thereof; and optionally

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- a further antibody for binding to the peptide, antibody or fragment thereof or the non ATP -binding P2X7 receptor, monomer or fragment thereof;

- written instructions for use of the kit in a method described above.

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[0016] In other instances there is provided a pharmaceutical composition including an antibody or fragment thereof as described above, or a peptide as described above together with a pharmaceutically acceptable carrier, diluent or excipient.

[0017] In related instances there is provided a method of treatment of a disease characterised by the expression of a non ATP -binding P2X7 receptor, monomer or fragment thereof including the step of providing an antibody or fragment thereof as described above, or a peptide as described above to an individual requiring said treatment.

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Detailed description of the instances

[0018] For the following descriptions, the technical and scientific terms used herein will have the meanings commonly understood by one of ordinary skill in the art, unless specifically defined otherwise.

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[0019] The inventors have identified an epitope that is exclusively expressed on non ATP - binding P2X7 receptors (otherwise known as "non functional receptors"). The epitope and peptides including the epitope have been found to be useful for generating antibodies and immune complexes that indicate the presence or absence or predisposition to a variety of diseases and conditions including carcinoma.

[0020] Thus in certain instances there is provided a peptide consisting of the sequence:

SEQ ID NO: 2: KYKKNVVEKRTLKVF

5 [0021] The peptide may consist of a sequence within the sequence of SEQ ID NO: 2 (Figure 2), in which case the peptide can be used as an immunogen to generate an antibody that is capable of selectively binding to a non ATP-binding P2X7 ("non-functional") receptor but not to an ATP-binding P2X7 ("functional") receptor. The term "non ATP-binding" and "non-functional" in relation to P2X7 may be used interchangeably in the specification and claims. Similarly, the term "ATP-binding" and "functional" may be used interchangeably in the specification and claims.

10 [0022] Generally a non ATP-binding receptor means a trimer or higher multimer formed from at least one P2X7 monomer, the trimer or higher multimer being at least substantially devoid of ATP binding activity. In certain instances, these trimers or higher multimers are substantially unable to form a pore in a cell membrane for ingress of calcium cations into the cell cytoplasm.

15 [0023] Methods for screening peptides that can be used as an immunogen to generate an antibody that is capable of selectively binding to a non ATP-binding P2X7 receptor but not to an ATP-binding P2X7 receptor are disclosed herein. One example includes the use of erythrocytes in a rosetting assay. In this assay an antibody that binds to functional receptors is used as a positive control in which rosettes are observed. A test antibody is determined not to bind to non functional receptors if it fails to form rosettes. It is determined to bind to non functional receptors if it is observed to bind to a non functional receptor- expressing cell line, including those discussed herein.

20 [0024] In these instances, the peptide may have a length of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17 residues.

[0025] In other instances the peptide consists of a sequence of SEQ ID NO: 3 (Figure 3), said peptide being useful as an immunogen to generate an antibody that is capable of selectively binding to a non ATP-binding P2X7 receptor but not to an ATP-binding P2X7 receptor.

25 [0026] The peptide may consist of a sequence within the sequence of SEQ ID NO: 3, said peptide being useful as an immunogen to generate an antibody that is capable of selectively binding to a non ATP-binding P2X7 receptor but not to an ATP-binding P2X7 receptor. Examples of these peptides include those having a sequence described in Table 1 (numbering according to SEQ ID NO: 1):

Table 1

30	K281 to K297	Y298 to G314
	T282 to Y298	Y299 to I315
	T283 to Y299	K300 to R316
35	N284 to K300	E301 to F317
	V285 to E301	N302 to D318
	S286 to N302	N303 to I319
	L287 to N303	V304 to L320
40	Y288 to V304	E305 to V321
	P289 to E305	K306 to F322
	G290 to K306	R307 to G323
45	Y291 to R307	T308 to T324
	N292 to T308	L309 to G325
	F293 to L309	I310 to G326
	R294 to I310	K311 to K327
50	Y295 to K311	V312 to F328
	A296 to V312	F313 to D329
	K297 to F313 (SEQ ID NO: 2)	

55 [0027] The peptide shown in Table 1 may have a length of from 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17 residues.

[0028] In other instances there is provided an antibody or fragment thereof capable of binding to a peptide described above.

[0029] The antibody may be produced by immunisation with a peptide as described above. An example of a suitable immunisation is described in Example 1 below.

[0030] The antibody may also be produced by immunisation with a non ATP-binding P2X7 receptor, such as a receptor having an amino acid sequence shown in SEQ ID NO: 1, or a fragment thereof including the amino acid sequences SEQ ID NOS: 3 to 9 (shown in Figures 3 to 9).

[0031] In one instance, the non ATP-binding P2X7 receptor, monomer or fragment thereof used for the immunisation has Pro210 in *cis* isomerisation.

[0032] The antibody may bind to an epitope that includes one or more residues of a peptide having a sequence of SEQ ID NO: 2. In certain instances, the antibody binds to K297 or Y298 or Y299 or K300 or E301 or N302 or N303 or V304 or E305 or K306 or R307 or T308 or L309 or 1310 or K311 or V312 or F313

[0033] The epitope may include a sequence of residues of a peptide having a sequence of SEQ ID NO: 2. In certain instances, the antibody binds to a sequence including K297 and Y298, or Y298 and Y299, or Y299 and K300, or K300 and E301, or E301 and N302, or N302 and N303, or N303 and V304, or V304 and E305, or E305 and K306, or K306 and R307, or R307 and T308, or T308 and L309, or L309 and 1310, or 1310 and K311, or K311 and V312, or V312 and F313.

[0034] The antibody that binds to one or more of the above residues may also bind to one or more residues of the P2X7 receptor extra cellular domain that are located outside of the region defined by SEQ ID NO:3. For example, the one or more residues located outside of the region defined by SEQ ID NO:3 may be located in a sequence of amino acid residues of an ATP-binding P2X7 receptor that defines the ATP binding site of the ATP-binding P2X7 receptor.

[0035] In one instance, the one or more residues located outside the sequence of SEQ ID NO:3 may be located in the sequence of SEQ ID NOS: 10 to 12 (Figures 10 to 12 respectively).

[0036] The antibody may be a whole antibody of any isotype. The antibody may be one obtained from monoclonal or polyclonal antisera. The antibody may be produced by hybridoma, or by recombinant expression.

[0037] The antibody may be chimeric, i.e. one containing human variable domains and non human constant domains. Alternatively, it may be humanized, i.e. one formed by grafting non human CDRs onto a human antibody framework. Still further, the antibody may be fully human.

[0038] The antibodies of the disclosure may be modified with respect to effector function, so as to enhance, e.g., the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) can be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated can have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). Homodimeric antibodies with enhanced anti-tumor activity can also be prepared using heterobifunctional crosslinkers. Alternatively, an antibody can be engineered that has dual Fc regions and can thereby have enhanced complement lysis and ADCC capabilities.

[0039] Where the antibody is an antibody fragment, the antibody fragment is selected from the group consisting of a dAb, Fab, Fd, Fv, F(ab')₂, scFv and CDR.

[0040] The antibody or fragment may be provided on a solid phase such as a bead, surface or tissue culture vessel.

[0041] The antibody or fragment thereof may be provided with a label for detection of binding of the antibody or fragment thereof to antigen.

[0042] The antibodies and fragments may be labelled for use in medical imaging. Such methods involve chemical attachment of a labelling or imaging agent, such as a radioisotope, which include ⁶⁷Cu, ⁹⁰Y, ¹²⁴I, ¹²⁵I, ¹³¹I, ¹⁸⁶Re, ¹⁸⁸Re, ²¹¹At, ²¹²Bi, administration of the labelled antibody or fragment to a subject in an acceptable carrier, and imaging the labelled antibody or fragment *in vivo* at the target site. Radio-labelled antibodies or fragments thereof may be particularly useful in *in vivo* imaging of cancers described herein.

[0043] The antibodies can be purified by methods known to the skilled artisan. Purification methods include, among other, selective precipitation, liquid chromatography, HPLC, electrophoresis, chromatofocusing, and various affinity techniques.

[0044] In some instances, the antibodies disclosed herein may also include multimeric forms of antibodies. For example, antibodies of the disclosure may take the form of antibody dimers, trimers, or higher-order multimers of monomeric immunoglobulin molecules.

[0045] Crosslinking of antibodies can be done through various methods known in the art. For example, crosslinking of antibodies may be accomplished through natural aggregation of antibodies, through chemical or recombinant linking techniques or other methods known in the art. For example, purified antibody preparations can spontaneously form protein aggregates containing antibody homodimers, and other higher-order antibody multimers. In a specific instance, crosslinking of antibodies by using a second antibody to bind to the antibodies of interest can be used to form a homodimer. The crosslinker antibody can be derived from a different animal compared to the antibody of interest. For example, a goat anti-mouse antibody (Fab specific) may be added to a mouse monoclonal antibody to form a homodimer. This bivalent crosslinker antibody recognizes the Fab or Fc region of the two antibodies of interest forming a homodimer.

[0046] Alternatively, antibody homodimers may be formed through chemical linkage techniques known in the art.

Chemical crosslinkers can be homo or heterobifunctional and will covalently bind with two antibodies forming a homodimer. In some instances, it is desirable that the chemical crosslinker not interact with the antigen-binding region of the antibody as this may affect antibody function. As will be appreciated by those skilled in the art, antibodies can be crosslinked at the Fab region.

5 **[0047]** In one instance there is provided an immune complex formed from the binding of an antibody or fragment thereof as described above to a non ATP -binding (i.e. non-functional) P2X7 receptor, monomer or fragment thereof. In one instance there is provided an immune complex formed from the binding of an antibody or fragment thereof to a peptide described above.

10 **[0048]** Generally an immune complex otherwise known as an antigen-antibody complex is a product that is formed from the binding of an antibody via an antibody binding site to an epitope on an antigen against which the antibody is raised. The complex may or may not consist of more than one antibody.

[0049] The immune complex is particularly important as detection of this *in vitro* or *in vivo* is indicative of presence of, or predisposition to a disease or condition including preneoplasia and neoplasia. These detection methods are described in more detail below.

15 **[0050]** The non-ATP binding P2X7 receptor, monomer or fragment thereof included in the immune complex may have Pro210 in *cis* isomerisation.

[0051] The non-ATP binding P2X7 receptor, monomer or fragment thereof included in the immune complex may have an amino acid sequence of any one of SEQ ID NOS: 4 to 9 or fragments thereof.

20 **[0052]** The non-ATP binding P2X7 receptor, monomer or fragment thereof included in the immune complex may have a molecular weight in the range of from about 15 to 80 kDa.

[0053] The non-ATP binding P2X7 receptor, monomer or fragment thereof included in the immune complex may lack a transmembrane domain.

25 **[0054]** The immune complex may be formed by binding a non-ATP binding P2X7 receptor, monomer or fragment thereof located on a cell surface membrane, in a cytoplasm, in a nucleus or in extra-cellular fluid. The extra -cellular fluid may be blood, plasma, serum, lymph, urine, semen, saliva, sputum, ascites, faeces, uterine and vaginal secretions, bile, amniotic fluid, cerebrospinal fluid and organ and tissue flushings.

[0055] The antibody or antibody fragment included in the immune complex may be attached to a solid phase, such as a bead or a plate, so that the immune complex is attached to a solid phase when formed. Alternatively, the non-ATP binding P2X7 receptor, monomer or fragment thereof included in the immune complex may be attached to a solid phase.

30 **[0056]** The antibody may be labelled for detection of formation of the immune complex.

[0057] The immune complex may further include an antibody or fragment thereof, such as a capture antibody for capture of the immune complex. The further antibody or fragment thereof may bind to the anti P2X7 receptor antibody. Also, the further antibody or fragment thereof may bind to the receptor or fragment thereof.

[0058] The further antibody or fragment thereof may be bound to a solid phase such as a phase described above.

35 **[0059]** The further antibody may be labelled for detection of formation of the immune complex. Examples of labels include fluorophores, dyes, isotopes etc.

[0060] In certain instances there is provided a method for determining whether a cell, tissue or extra cellular body fluid includes a non ATP -binding P2X7 receptor, monomer or fragment thereof including:

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- contacting a cell, tissue or extra cellular body fluid with an antibody or fragment thereof in conditions for forming an immune complex as described above, and
 - detecting whether an immune complex has been formed,

45 wherein detection of an immune complex determines that a cell, tissue or extra -cellular body fluid includes a non ATP -binding P2X7 receptor, monomer or fragment thereof.

[0061] In other instances there is provided a use of an antibody or fragment thereof described above in the manufacture of means for determining whether a cell, tissue or extra-cellular body fluid contains a P2X7 receptor, monomer or fragment thereof.

50 **[0062]** In other instances there is provided a method for determining whether a cell, tissue or extra -cellular body fluid contains an antibody against a non ATP -binding P2X7 receptor, monomer or fragment thereof including:

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- contacting a cell, a tissue or an extra -cellular body fluid with a peptide as described above in conditions for forming an immune complex between the peptide and an antibody in the cell, tissue or extra -cellular body fluid, and
 - detecting whether an immune complex has been formed,

wherein detection of an immune complex determines that a cell, tissue or extra -cellular body fluid contains an antibody

against a non ATP -binding P2X7 receptor, monomer or fragment thereof.

[0063] In other instances there is provided a use of a peptide described above in the manufacture of means for determining whether a cell, tissue or extra-cellular body fluid contains an antibody against a non ATP -binding P2X7 receptor, monomer or fragment thereof.

[0064] The presence of a given protein, or level of expression of a given protein in a host cell, tissue or extra -cellular body fluid can be detected by any number of assays. Examples include immunoassays, chromatography and mass spectrometry.

[0065] Immunoassays, i.e. assays involving an element of the immune system are particularly preferred. These assays may generally be classified into one of:

(i) assays in which purified antigen is used to detect an antibody in host serum. For example, purified antigen is bound to solid phase by adsorption or indirectly through another molecule and host serum is applied followed by another antibody for detecting presence or absence of host antibody;

(ii) assays in which purified antigen is used to detect immune cells, such as T and B lymphocytes. For example, peripheral white cells are purified from a host and cultured with purified antigen. The presence or absence of one or factors indicating immunity are then detected. Other examples include assays that measure cell proliferation (lymphocyte proliferation or transformation assays) following exposure to purified antigen, and assays that measure cell death (including apoptosis) following exposure to purified antigen;

(iii) assays in which purified antibody specific for antigen is used to detect antigen in the host. For example, purified antibody is bound to solid phase, host tissue is then applied followed by another antibody specific for the antigen to be detected. There are many examples of this approach including ELISA, RIA;

(iv) assays in which a purified anti-idiotypic antibody is used to detect host antibody. For example, anti-idiotypic antibody is adsorbed to solid phase, host serum is added and anti-Fc antibody is added to bind to any host antibodies having been bound by the anti-idiotypic antibody.

[0066] The immunoassays can be applied *in vitro* or *in vivo*.

[0067] In one instance , the disease is typically a cancer such as carcinoma, sarcoma, lymphoma, or leukemia. Carcinomas that may be detected include, but not limited to, prostate, breast, skin, lung, cervix, uterus, stomach, oesophagus, bladder, and colon cancers.

[0068] Whilst any body fluid can be used to detect any of these diseases, some body fluids may be more appropriate than others to detect certain diseases, for example urine may be more appropriate to detect prostate cancer and blood for detecting blood cancers such as lymphoma.

[0069] In another instance there is provided a method for determining whether an individual has a cancer including the steps of:

- contacting a cell, a tissue or an extra -cellular body fluid with a peptide as described above in conditions for forming an immune complex between the peptide and an antibody in the cell, tissue or extra -cellular body fluid; or
- contacting a cell, tissue or extra cellular body fluid with an antibody or fragment thereof in conditions for forming an immune complex as described above, and
- detecting whether an immune complex has been formed,

wherein detection of an immune complex determines that an individual has a cancer.

[0070] In a further instance there is provided use of anti purinergic (P2X) receptor antibody or fragment thereof as described above, or a peptide as described above for determining whether an individual has a cancer.

[0071] In certain instances , cancer is selected from the group consisting of prostate cancer, invasive breast cancer, melanoma, adenocarcinoma of the bowel, serous ovarian cancer, squamous cell cancer of the cervix, endometrial cancer, small cell lung cancer, hepatocellular carcinoma, transitional cell carcinoma of the bladder, gastrointestinal stromal tumour, endometrial stromal tumour, pituitary cancer, mesothelioma, Hodgkin's lymphoma and thyroid papillary.

[0072] In yet further instances there is provided a kit or composition for determining whether a cell, tissue or extra -cellular body fluid contains a non ATP -binding P2X7 receptor, monomer or fragment thereof, or an antibody against same including:

- a peptide as described above; and/or

- an antibody or fragment thereof as described above; and/or
- non ATP -binding P2X7 receptor, monomer or fragment thereof; and optionally
- 5 - a further antibody for binding to the peptide, antibody or fragment thereof or the non ATP -binding P2X7 receptor, monomer or fragment thereof;
- written instructions for use of the kit in a method described above.

10 **[0073]** Kits are provided which contain the necessary reagents to carry out the assays of the present disclosure. The kit may include one or more compartments, each to receive one or more containers such as: (a) a first container comprising one of the components of the present disclosure described above; and (b) one or more other containers comprising one or more of the following: wash reagents, reagents capable of detecting presence of the antibody or peptide.

15 **[0074]** The containers allow one to efficiently transfer reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated, and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another.

20 **[0075]** The kit typically contains containers which may be formed from a variety of materials such as glass or plastic, and can include for example, bottles, vials, syringes, and test tubes. A label typically accompanies the kit, and includes any writing or recorded material, which may be electronic or computer readable form (e.g., disk, optical disc, or tape) providing instructions or other information for used of the contents of the kit. The label indicates that the formulation is used for diagnosing or treating the disorder of choice.

[0076] One skilled in the art will readily recognize that the disclosed antibodies and peptides of the present disclosure can be readily incorporated into one of the established kit formats which are well known in the art.

25 **[0077]** In other instances there is provided a pharmaceutical composition including an antibody or fragment thereof as described above, or a peptide as described above, together with a pharmaceutically acceptable carrier, diluent or excipient.

30 **[0078]** In the preparation of the pharmaceutical compositions comprising the antibodies or peptides described in the teachings herein, a variety of vehicles and excipients and routes of administration may be used, as will be apparent to the skilled artisan. Representative formulation technology is taught in, inter alia, Remington: The Science and Practice of Pharmacy, 19th Ed., Mack Publishing Co., Easton, PA (1995) and Handbook of Pharmaceutical Excipients, 3rd Ed, Kibbe, A.H. ed., Washington DC, American Pharmaceutical Association (2000).

[0079] The pharmaceutical compositions will generally comprise a pharmaceutically acceptable carrier and a pharmacologically effective amount of the antibodies or peptides, or mixture of antibodies or mixture of peptides, or suitable salts thereof.

35 **[0080]** The pharmaceutical composition may be formulated as powders, granules, solutions, suspensions, aerosols, solids, pills, tablets, capsules, gels, topical creams, suppositories, transdermal patches, and other formulations known in the art.

40 **[0081]** For the purposes described herein, pharmaceutically acceptable salts of the antibodies and peptides is intended to include any art recognized pharmaceutically acceptable salts including organic and inorganic acids and/or bases. Examples of salts include sodium, potassium, lithium, ammonium, calcium, as well as primary, secondary, and tertiary amines, esters of lower hydrocarbons, such as methyl, ethyl, and propyl. Other salts include organic acids, such as acetic acid, propionic acid, pyruvic acid, maleic acid, succinic acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, salicylic acid, etc.

45 **[0082]** As used herein, "pharmaceutically acceptable carrier" comprises any standard pharmaceutically accepted carriers known to those of ordinary skill in the art in formulating pharmaceutical compositions. Thus, the antibodies or peptides, by themselves, such as being present as pharmaceutically acceptable salts, or as conjugates, may be prepared as formulations in pharmaceutically acceptable diluents; for example, saline, phosphate buffer saline (PBS), aqueous ethanol, or solutions of glucose, mannitol, dextran, propylene glycol, oils (e.g., vegetable oils, animal oils, synthetic oils, etc.), microcrystalline cellulose, carboxymethyl cellulose, hydroxypropyl methyl cellulose, magnesium stearate, calcium phosphate, gelatin, polysorbate 80 or as solid formulations in appropriate excipients.

50 **[0083]** The pharmaceutical compositions will often further comprise one or more buffers (e.g., neutral buffered saline or phosphate buffered saline), carbohydrates (e.g., glucose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants (e.g., ascorbic acid, sodium metabisulfite, butylated hydroxytoluene, butylated hydroxyanisole, etc.), bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (e.g., aluminium hydroxide), solutes that render the formulation isotonic, hypotonic or weakly hypertonic with the blood of a recipient, suspending agents, thickening agents and/or preservatives. Alternatively, compositions of the present disclosure may be formulated as a lyophilizate.

55 **[0084]** While any suitable carrier known to those of ordinary skill in the art may be employed in the compositions of

this disclosure, the type of carrier will typically vary depending on the mode of administration. Antibody and peptide compositions may be formulated for any appropriate manner of administration, including for example, oral, nasal, mucosal, intravenous, intraperitoneal, intradermal, subcutaneous, and intramuscular administration.

5 **[0085]** For parenteral administration, the compositions can be administered as injectable dosages of a solution or suspension of the substance in a physiologically acceptable diluent with a pharmaceutical carrier that can be a sterile liquid such as sterile pyrogen free water, oils, saline, glycerol, polyethylene glycol or ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, surfactants, pH buffering substances and the like can be present in compositions.

10 **[0086]** Other components of pharmaceutical compositions are those of petroleum, animal, vegetable, or synthetic origin, for example, non-aqueous solutions of peanut oil, soybean oil, corn oil, cottonseed oil, ethyl oleate, and isopropyl myristate. Antibodies and peptides can be administered in the form of a depot injection or implant preparation which can be formulated in such a manner as to permit a sustained release of the active ingredient. An exemplary composition comprises antibody at 5 mg/ml, formulated in aqueous buffer consisting of 50 mM L-histidine, 150 mM NaCl, adjusted to pH 6.0 with HCl.

15 **[0087]** Typically, the compositions are prepared as injectables, either as liquid solutions or suspensions; solid or powder forms suitable for reconstitution with suitable vehicles, including by way example and not limitation, sterile pyrogen free water, saline, buffered solutions, dextrose solution, etc., prior to injection. The preparation also can be emulsified or encapsulated in liposomes or micro particles such as polylactide, polyglycolide, or copolymers.

20 **[0088]** The pharmaceutical compositions described herein may be presented in unit-dose or multi-dose containers, such as sealed ampoules or vials. Such containers are typically sealed in such a way to preserve the sterility and stability of the formulation until use. In general, formulations may be stored as suspensions, solutions or emulsions in oily or aqueous vehicles, as indicated above.

[0089] Alternatively, a pharmaceutical composition may be stored in a freeze-dried condition requiring only the addition of a sterile liquid carrier immediately prior to use.

25 **[0090]** In related instances there is provided a method of treatment of a disease characterised by the expression of a non ATP-binding P2X7 receptor, monomer or fragment thereof including the step of providing an antibody or fragment thereof as described above, or a peptide as described above to an individual requiring said treatment.

30 **[0091]** Methods of immunotargeting cancer cells using antibodies or antibody fragments are well known in the art. U.S. Pat. No. 6,306,393 describes the use of anti-CD22 antibodies in the immunotherapy of B-cell malignancies, and U.S. Pat. No. 6,329,503 describes immunotargeting of cells that express serpentine transmembrane antigens. Antibodies described herein (including humanized or human monoclonal antibodies or fragments or other modifications thereof, optionally conjugated to cytotoxic agents) can be introduced into a patient such that the antibody binds to cancer cells and mediates the destruction of the cells and the tumor and/or inhibits the growth of the cells or the tumor.

35 **[0092]** Without intending to limit the disclosure, mechanisms by which such antibodies can exert a therapeutic effect may include complement-mediated cytolysis, antibody-dependent cellular cytotoxicity (ADCC)1 modulating the physiologic function of the tumor antigen, inhibiting binding or signal transduction pathways, modulating tumor cell differentiation, altering tumor angiogenesis factor profiles, modulating the secretion of immune stimulating or tumor suppressing cytokines and growth factors, modulating cellular adhesion, and/or by inducing apoptosis.

40 **[0093]** The antibodies can also be conjugated to toxic or therapeutic agents, such as radioligands or cytosolic toxins, and may also be used therapeutically to deliver the toxic or therapeutic agent directly to tumor cells.

45 **[0094]** By "treatment" herein is meant therapeutic or prophylactic treatment, or a suppressive measure for the disease, disorder or undesirable condition. Treatment encompasses administration of the subject antibodies in an appropriate form prior to the onset of disease symptoms and/or after clinical manifestations, or other manifestations, of the disease to reduce disease severity, halt disease progression, or eliminate the disease. Prevention of the disease includes prolonging or delaying the onset of symptoms of the disorder or disease, preferably in a subject with increased susceptibility to the disease.

50 **[0095]** The therapeutic preparations can use nonmodified antibodies or antibodies conjugated with a therapeutic compound, such as a toxin or cytotoxic molecule, depending on the functionality of the antibody. Generally, when non-modified antibodies are used, they will typically have a functional Fc region. By "functional Fc region" herein is meant a minimal sequence for effecting the biological function of Fc, such as binding to Fc receptors, particularly Fc γ R (e.g., Fc γ R1, α RIIA, RIIB, RIIIA, RIIIB).

55 **[0096]** Without being bound by theory, it is believed that the Fc region may affect the effectiveness of anti-tumor monoclonal antibodies by binding to Fc receptors immune effector cells and modulating cell mediated cytotoxicity, endocytosis, phagocytosis, release of inflammatory cytokines, complement mediated cytotoxicity, and antigen presentation. In this regard, polyclonal antibodies, or mixtures of monoclonals will be advantageous because they will bind to different epitopes and thus have a higher density of Fc on the cell surface as compared to when a single monoclonal antibody is used. Of course, to enhance their effectiveness in depleting targeted cells, or where nonmodified antibodies are not therapeutically effective, antibodies conjugated to toxins or cytotoxic agents may be used.

[0097] The antibody compositions may be used either alone or in combination with other therapeutic agents to increase efficacy of traditional treatments or to target abnormal cells not targeted by the antibodies. Combining the antibody therapy method with a chemotherapeutic, radiation or surgical regimen may be preferred in patients that have not received chemotherapeutic treatment, whereas treatment with the antibody therapy may be indicated for patients who have received one or more chemotherapies. Additionally, antibody therapy can also enable the use of reduced dosages of concomitant chemotherapy, particularly in patients that do not tolerate the toxicity of the chemotherapeutic agent very well. Furthermore, treatment of cancer patients with the antibody with tumors resistant to chemotherapeutic agents might induce sensitivity and responsiveness to these agents in combination.

[0098] In one aspect, the antibodies are used adjunctively with therapeutic cytotoxic agents, including, by way of example and not limitation, busulfan, thioguanine, idarubicin, cytosine arabinoside, 6-mercaptopurine, doxorubicin, daunorubicin, etoposide, and hydroxyurea. Other agents useful as adjuncts to antibody therapy are compounds directed specifically to the abnormal cellular molecule found in the disease state. These agents will be disease specific. For example, for treating chronic myeloid leukemia arising from BCR-ABL activity, one class of useful compounds are inhibitors of abl kinase activity, such as Imatinib, an inhibitor of bcr-abl kinase, and antisense oligonucleotides against bcr (e.g., Oblimersen). Other agents include, among others, interferon-alpha, humanized anti- CD52, deacetylase inhibitor FR901228 (depsipeptide), and the like.

[0099] The amount of the compositions needed for achieving a therapeutic effect will be determined empirically in accordance with conventional procedures for the particular purpose. Generally, for administering the compositions ex vivo or in vivo for therapeutic purposes, the compositions are given at a pharmacologically effective dose. By "pharmacologically effective amount" or "pharmacologically effective dose" is an amount sufficient to produce the desired physiological effect or amount capable of achieving the desired result, particularly for treating or retreating the disorder or disease condition, including reducing or eliminating one or more symptoms or manifestations of the disorder or disease.

[0100] As an illustration, administration of antibodies to a patient suffering from prostate cancer provides a therapeutic benefit not only when the underlying disease is eradicated or ameliorated, but also when the patient reports a decrease in the severity or duration of the symptoms associated with the disease. Therapeutic benefit also includes halting or slowing the progression of the underlying disease or disorder, regardless of whether improvement is realized.

[0101] The amount administered to the host will vary depending upon what is being administered, the purpose of the administration, such as prophylaxis or therapy, the state of the host, the manner of administration, the number of administrations, interval between administrations, and the like. These can be determined empirically by those skilled in the art and may be adjusted for the extent of the therapeutic response. Factors to consider in determining an appropriate dose include, but is not limited to, size and weight of the subject, the age and sex of the subject, the severity of the symptom, the stage of the disease, method of delivery of the agent, half-life of the agents, and efficacy of the agents. Stage of the disease to consider includes whether the disease is acute or chronic, relapsing or remitting phase, and the progressiveness of the disease. Determining the dosages and times of administration for a therapeutically effective amount are well within the skill of the ordinary person in the art.

[0102] For any compositions of the present disclosure, the therapeutically effective dose is readily determined by methods well known in the art. For example, an initial effective dose can be estimated from cell culture or other in vitro assays. For example, Sliwkowsky, MX et al., Semin. Oncol. 26.suppl. 12) 60-70 (1999) describes in vitro measurements of antibody dependent cellular cytotoxicity. A dose can then be formulated in animal models to generate a circulating concentration or tissue concentration, including that of the IC50 as determined by the cell culture assays.

[0103] In addition, the toxicity and therapeutic efficacy are generally determined by cell culture assays and/or experimental animals, typically by determining a LD50 (lethal dose to 50% of the test population) and ED50 (therapeutically effectiveness in 50% of the test population). The dose ratio of toxicity and therapeutic effectiveness is the therapeutic index. Preferred are compositions, individually or in combination, exhibiting high therapeutic indices. Determination of the effective amount is well within the skill of those in the art, particularly given the detailed disclosure provided herein. Guidance is also found in standard reference works, for example Fingl and Woodbury, General Principles In: The Pharmaceutical Basis of Therapeutics pp. 1-46 (1975), and the references cited therein.

[0104] To achieve an initial tolerizing dose, consideration is given to the possibility that the antibodies may be immunogenic in humans and in non-human primates. The immune response may be biologically significant and may impair the therapeutic efficacy of the antibody even if the antibody is partly or chiefly comprised of human immunoglobulin sequences such as, for example, in the case of a chimeric or humanized antibody. Within certain instances, an initial high dose of antibody is administered such that a degree of immunological tolerance to the therapeutic antibody is established.

[0105] The tolerizing dose is sufficient to prevent or reduce the induction of an antibody response to repeat administration of the committed progenitor cell specific antibody.

[0106] Preferred ranges for the tolerizing dose are between 10 mg/kg body weight to 50 mg/kg body weight, inclusive. More preferred ranges for the tolerizing dose are between 20 and 40 mg/kg, inclusive. Still more preferred ranges for the tolerizing dose are between 20 and 25 mg/kg, inclusive.

[0107] Within these therapeutic regimens, the therapeutically effective dose of antibodies is preferably administered in the range of 0.1 to 10 mg/kg body weight, inclusive. More preferred second therapeutically effective doses are in the range of 0.2 to 5 mg/kg body weight, inclusive. Still more preferred therapeutically effective doses are in the range of 0.5 to 2 mg/kg, inclusive. Within alternative instances, the subsequent therapeutic dose or doses may be in the same or different formulation as the tolerizing dose and/or may be administered by the same or different route as the tolerizing dose.

[0108] For the purposes of this disclosure, the methods of administration are chosen depending on the condition being treated, the form of the subject antibodies, and the pharmaceutical composition.

[0109] Administration of the antibody compositions can be done in a variety of ways, including, but not limited to, continuously, subcutaneously, intravenously, orally, topically, transdermal, intraperitoneal, intramuscularly, and intravesically. For example, microparticle, microsphere, and microencapsulate formulations are useful for oral, intramuscular, or subcutaneous administrations. Liposomes and nanoparticles are additionally suitable for intravenous administrations. Administration of the pharmaceutical compositions may be through a single route or concurrently by several routes. For instance, intraperitoneal administration can be accompanied by intravenous injections. Preferably the therapeutic doses are administered intravenously, intraperitoneally, intramuscularly, or subcutaneously.

[0110] The compositions may be administered once or several times. In some instances, the compositions may be administered once per day, a few or several times per day, or even multiple times per day, depending upon, among other things, the indication being treated and the judgement of the prescribing physician.

[0111] Administration of the compositions may also be achieved through sustained release or long-term delivery methods, which are well known to those skilled in the art. By "sustained release or" "long term release" as used herein is meant that the delivery system administers a pharmaceutically therapeutic amount of subject compounds for more than a day, preferably more than a week, and most preferable at least about 30 days to 60 days, or longer. Long term release systems may comprise implantable solids or gels containing the antibodies, such as biodegradable polymers described above; pumps, including peristaltic pumps and fluorocarbon propellant pumps; osmotic and mini-osmotic pumps; and the like.

[0112] The method of the disclosure contemplates the administration of single monoclonal antibodies and any antibody that recognizes the particular antigens recognized by these antibodies, as well as combinations, of different mAbs. Two or more monoclonal antibodies may provide an improved effect compared to a single antibody. Alternatively, a combination of an antibody with an antibody that binds a different antigen may provide an improved effect compared to a single antibody. Such mAb cocktails may have certain advantages inasmuch as they contain mAbs, which exploit different effector mechanisms or combine directly cytotoxic mAbs with mAbs that rely on immune effector functionality. Such mAbs in combination may exhibit synergistic therapeutic effects.

[0113] It will be understood that the disclosure disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the disclosure.

[0114] The following protocols are provided as non-limiting examples for the purpose of illustrating the invention.

Examples

Example 1

[0115] Anti-nonfunctional P2X₇ antibody was raised against the peptide KYYKENNVEKRTLKVF (SEQ ID NO: 2) or Lys-Tyr-Tyr-Lys-Glu-Asn-Asn-Val-Glu-Lys-Arg-Thr-Leu-Ile-Lys-Val-Phe, representing amino acids 297-313 of the human P2X₇ protein. The peptide was synthesized by solid phase chemistry (Mimotopes Pty Ltd, Melbourne, Australia) to high purity (>95%) as judged by mass spectroscopy. A C-terminal Cys (C) residue was attached to the sequence and to this was attached the cross-linker MCS (6-Maleimido-Caproic Acid N-Hydroxysuccinimide Ester) for conjugating the peptide to carrier proteins including separately Diphtheria toxoid, bovine serum albumin and ovalbumin by Mimotopes.

Example 2

[0116] Female New Zealand White rabbits, aged between 10-12 weeks, were immunized according to the following schedule.

[0117] On day one, each rabbit received 200 ug of P2X₇ epitope conjugated to diphtheria toxoid (total antigen weight ~ 500 ug of conjugated epitope). This conjugated peptide was supplied by Mimotopes Pty Ltd as a stable solution.

[0118] The epitope conjugate was diluted in sterile PBS to a concentration of 500 ug per 0.8 ml PBS. To this was added 0.1ml of DEAE/Dextran/QUILA™ solution (2.5 mg QUILA™ plus 25 mg DEAE/Dextran per mL of PBS) and 1.2 mL of Montanide 15A50V. This solution was emulsified using glass luerloc syringes and a narrow bore luerloc coupling.

[0119] Animals were each injected with 2 ml of the epitope adjuvant emulsion at multiple subcutaneous and intra-

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muscular sites.

[0120] The above was repeated at 6-weeks and 9-weeks post initial injections. At week 10, the rabbits were injected intravenously with 1 ml of sterile PBS containing 50 ug of DT-conjugated P2X₇ epitope. Four days later, the rabbits were bled out and the serum containing the antibody was stored for future analysis and use in immunoassays.

Example 3

[0121] The procedure in Example 2 was used except that mice were injected with 20 ug of P2X₇ epitope (~50 ug of DT-conjugate) in 0.2 mL of epitope adjuvant emulsion.

[0122] Four days after the intravenous injection, antibody titers were measured in mouse blood and the highest titer mice were selected as spleen donors for hybridoma fusions.

Example 4

[0123] Mice selected above were used as spleen cell donors and these cells were fused with mouse SP20 myeloma cells to form hybridoma cell lines according to the 96-well plate format modification of the original protocol described by Kohler and Milstein.

[0124] Cell lines were selected for stability and production of the specific antibody to particular P2X₇ epitopes.

Example 5

[0125] A monoclonal antibody with suitably high affinity for the target epitope was selected for the IHC study. The binding characteristics of the antibody was tested by measuring the interaction with the target epitope on a Biacore instrument. A total of 550 resonance units of binding was achieved in the 60 second loading time showing slow on rate. A very slow off rate was apparent following cessation of loading with no measurable diminution of binding over the subsequent 10 minutes.

Example 6

[0126] Binding of the monoclonal antibody to fixed and permeabilized C11STH cells expressing the non-functional receptor was performed on a flow cytometer (Becton-Dickenson) using Alexa-488 label. The mean value was 90.8 compared with negative controls of 1.9. Binding of the monoclonal antibody to receptors on the surface of unfixed live cells was also assessed. The mean value was 5.8 compared with 0.34 for the negative control.

Example 7

[0127] For fixed cells, standard fluorescent antibody staining and confocal microscopy was used as follows. Fixed cells on poly-L-lysine-coated glass coverslips in 48-well plates were incubated with 20% normal horse serum in phosphate buffered saline pH7.5 (PBS) for 20 minutes, washed with PBS for 5 minutes then incubated with primary antibody for 30 min, washed with PBS for 5 minutes and finally labeled with fluorescent labeled secondary antibody (Jackson Immunologics) for 30 minutes. Cells were then washed twice (2 x 5 minutes) with PBS before mounting the coverslips on slides in 50% glycerol in PBS. Cells were visualized with a Leica TCS NT UV laser confocal microscope system with the pinhole set at 1.0. Murine isotype control antibodies were used routinely as negative controls and showed no staining. Western blots of HEK293 cells transfected with P2X₇ revealed a single band at approximately 75 kDa that was absent in non-transfected cells and in samples of homogenates pre-treated with the epitope. Cell protein extracts (30 ug) together with molecular weight markers were fractionated on sodium dodecyl sulfate polyacrylamide gel (8-16%) (Novex). Proteins were electro-blotted on Immobilon-P membrane (Millipore). Western blots were developed using the ECL-chemiluminescence system (Amersham).

Example 8

[0128] A total of 25 different cases of breast cancers, 25 cases of skin cancers and 25 cases of prostate cancers were examined by immunohistochemistry. There was no cross-reactivity between the functional receptors present on the red blood cells and the non-functional receptor antibody (data not shown).

[0129] Examples of cancer tissue including prostate cancer, breast cancer as represented by ductal carcinoma in situ and melanoma all stained for non-functional P2X₇. The epithelium in normal tissues with no adjacent tumour was devoid of the receptor while all cancer cases stained for the receptor.

[0130] Cancer cell lines derived from these and other tissues and other were similarly found to express the receptors.

Examples tested and found positive include ADDP, PC3, LNCap, MCF7, MDA-MB-235, MDA-MB-431, NCIH460, NCIH69, NCIH596, DU145, ACHN, 786-O, Hep3B2, C11STH and BT474. Similar patterns of expression were seen on tumor xenografts of ovarian ADDP and lung NCIH69 respectively. The non-functional P2X₇ receptors were found on the plasma membrane with correspondingly little residual cytoplasmic receptor in the most advanced stages of the cancers. In contrast, lower grade tumours exhibited a preponderance of cells in which a large proportion of the receptors remain intracellular. Such a progressive transport of non-functioning apoptotic receptors to the plasma membrane over the course of the disease progression indicates that cancer cell lines should exhibit non-functional P2X₇ receptors on the plasma membrane rather than being largely intracellular.

[0131] Normal epithelial tissue is devoid of the non-functional receptors while cancer tissues are all labelled. Different breast cancers, including both invasive and *in situ* lobular and ductal carcinomas were examined. All types expressed non-functional receptors. Epithelial cells in these and other normal, non-cancerous tissues such as bowel, bladder, ovarian, uterine, cervical, stomach and lung were found to be devoid of non-functional receptors.

[0132] Areas of morphologically normal tissue surrounding tumours in prostate also expressed the receptors consistent with the field-effect in which tumour cells influence surrounding normal epithelium in connected ducts thereby alerting these cells to the presence of a developing tumour. In response, these cells begin deploying receptor, initially in an entirely intracellular location but eventually on the plasma membrane.

[0133] In addition to the breast, skin and prostate cancer samples, other examples of epithelial cell cancers, all of which expressed non-functional P2X₇ included bowel adenocarcinoma, invasive ovarian cancer, squamous cell carcinoma of the cervix, endometrial carcinoma of the uterus, small cell lung cancer, hepatocellular carcinoma, transitional cell carcinoma of the bladder and Barrett's mucosa with adenocarcinoma.

[0134] Human cancers of non-epithelial cell origin were also examined. All were found to express the non-functional P2X₇ receptors. Examples of mesenchymal cancers include gastrointestinal stromal tumour and endometrial stromal tumour. Equally, other tumours derived from this cell type such as Ewing's sarcoma express the receptors as do brain tumours such as oligodendrogliomas and astrocytomas, as do pituitary carcinoma. Mesothelioma, a cancer derived from pleural cells, also express non functional receptors, as do solid tumours derived from blood cells such as mantle cell lymphomas. Hodgkin's lymphoma and thyroid papillary are examples.

[0135] Cross-reactivity between human and other mammalian cancers such as prostate, breast and skin from dogs and cats and melanoma in mice was observed using the same antibody to human receptor. These observations add weight to the conclusion that the cancer cell target is ubiquitous.

Example 9

[0136] There are indications that the receptor expression alters with tumour grade providing the potential to differentiate between latent and aggressive forms of cancer. Certainly, very slow growing low grade prostate cancers exhibit a pattern of receptor expression that is almost entirely intracellular, while cases of invasive prostate cancer exhibit more plasma membrane and myoepithelial cell labelling together with a significantly elevated receptor density.

[0137] Low grade and clinically unimportant prostate cancer can be differentiated from clinically important prostate cancer by the appearance of the surrounding areas of normal epithelium. Clinically important cancers include those that are likely to spread into the prostatic stroma and metastasize. Direct sampling of the tumour with trans rectal ultrasound (TRUS) guided needle biopsies may detect a clinically significant tumour, thus making diagnosis straightforward. In cases in which the extant tumour is sampled in a region that is lower grade (eg Gleason 3+3) with other areas (eg Gleason 3+4) missed, staining for the non-functional P2X₇ receptor shows that areas of normal morphology also sampled in the prostate show intense levels of receptor expression and myoepithelial stain, indicating the presence of tissue that is metastasizing. Even cases in which the tumour is completely missed, such as tumours confined to the apical lobe out of reach of the needles, the presence and status of the tumour is readily detected as the field-effect emanating from the tumour to surrounding epithelium alerts the pathologist to the presence of the tumour and the likely grade.

Example 10

[0138] Dysplastic tissue is difficult to diagnose and conditions such as Barrett's mucosa with dysplasia need to be monitored given the propensity of the tissue to transform to adenocarcinoma. The differentiation between dysplastic tissue that will remain benign and tissue that is at extreme risk of imminent transformation again centres on the presence of associated myoepithelial stain showing a dramatic up-regulation of non-functional P2X₇ receptor expression. Similar results were observed for other dysplastic bowel conditions such as colitis. In tissue samples of Barrett's with dysplasia there was no staining indicating a benign condition while samples with intense myoepithelial stain identified a subject that had an associated adenocarcinoma.

[0139] Early cases of neoplastic transformation including cervical intraepithelial neoplasia (CIN) grades 1-3 also showed increased levels of receptor expression and cases such as ovarian serous borderline were categorised as being

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benign (unstained) and those that were going to transform (heavy epithelial stain).

[0140] The above results indicate that an epitope that includes one or more amino acids of the sequence KYKKN-NVEKRTLKVF has expression on a broad selection of epithelial cancer tissues and cells.

5 SEQUENCE LISTING

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25 Trp Met Asp Pro Gln Ser Lys Gly Ile Gln Thr Gly Arg Cys Val Val
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His Glu Gly Asn Gln Lys Thr Cys Glu Val Ser Ala Trp Cys Pro Ile
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30 Glu Ala Val Glu Glu Ala Pro Arg Pro Ala Leu Leu Asn Ser Ala Glu
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35 Asn Phe Thr Val Leu Ile Lys Asn Asn Ile Asp Phe Pro Gly His Asn
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Tyr Thr Thr Arg Asn Ile Leu Pro Gly Leu Asn Ile Thr Cys Thr Phe
 115 120 125

40 His Lys Thr Gln Asn Pro Gln Cys Pro Ile Phe Arg Leu Gly Asp Ile
 130 135 140

45 Phe Arg Glu Thr Gly Asp Asn Phe Ser Asp Val Ala Ile Gln Gly Gly
 145 150 155 160

Ile Met Gly Ile Glu Ile Tyr Trp Asp Cys Asn Leu Asp Arg Trp Phe
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50 His His Cys Arg Pro Lys Tyr Ser Phe Arg Arg Leu Asp Asp Lys Thr
 180 185 190

55 Thr Asn Val Ser Leu Tyr Pro Gly Tyr Asn Phe Arg Tyr Ala Lys Tyr
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55	Thr 465	Trp	Arg	Phe	Gly	Ser 470	Gln	Asp	Met	Ala	Asp 475	Phe	Ala	Ile	Leu	Pro 480			
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 5 Asn Phe Thr Val Leu Ile Lys Asn Asn Ile Asp Phe Pro Gly His Asn
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 Tyr Thr Thr Arg Asn Ile Leu Pro Gly Leu Asn Ile Thr Cys Thr Phe
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 10 His Lys Thr Gln Asn Pro Gln Cys Pro Ile Phe Arg Leu Gly Asp Ile
 50 55 60
 15 Phe Arg Glu Thr Gly Asp Asn Phe Ser Asp Val Ala Ile Gln Gly Gly
 65 70 75 80
 20 Ile Met Gly Ile Glu Ile Tyr Trp Asp Cys Asn Leu Asp Arg Trp Phe
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 100 105 110
 25 Thr Asn Val Ser Leu Tyr Pro Gly Tyr Asn Phe Arg Tyr Ala Lys Tyr
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 Ile Arg Phe Asp Ile Leu Val Phe Gly Thr Gly Gly Lys Phe Asp Ile
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 35 Ile Gln Leu Val Val Tyr Ile Gly Ser Thr Leu Ser Tyr Phe Gly Leu
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 40 Cys Arg Ser His Ile Tyr Pro Trp Cys Lys Cys Cys Gln Pro Cys Val
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 45 Val Asn Glu Tyr Tyr Tyr Arg Lys Lys Cys Glu Ser Ile Val Glu Pro
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 55

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5 Met Val Asn Gln Gln Leu Leu Gly Arg Ser Leu Gln Asp Val Lys Gly
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15 Pro Leu Ala Leu His Asp Thr Pro Pro Ile Pro Gly Gln Pro Glu Glu
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20 Ile Gln Leu Leu Arg Lys Glu Ala Thr Pro Arg Ser Arg Asp Ser Pro
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25 Val Trp Cys Gln Cys Gly Ser Cys Leu Pro Ser Gln Leu Pro Glu Ser
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30 His Arg Cys Leu Glu Glu Leu Cys Cys Arg Lys Lys Pro Gly Ala Cys
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45 Asp Ser Thr Asn Ser Arg Leu Arg His Cys Ala Tyr Arg Cys Tyr Ala
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Phe His Val Ile Ile Phe Ser Tyr Val Cys Phe Ala Leu Val Ser Asp
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 20 Leu Gln Gly Asn Ser Phe Phe Val Met Thr Asn Phe Leu Lys Thr Glu
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 40 Thr Cys Glu Val Ser Ala Trp Cys Pro Ile Glu Ala Val Glu Glu Ala
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 45 Pro Arg Pro Ala Leu Leu Asn Ser Ala Glu Asn Phe Thr Val Leu Ile
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 50 Lys Asn Asn Ile Asp Phe Pro Gly His Asn Tyr Thr Thr Arg Asn Ile
 195 200 205
 55 Leu Pro Gly Leu Asn Ile Thr Cys Thr Phe His Lys Thr Gln Asn Pro
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 60 Gln Cys Pro Ile Phe Arg Leu Gly Asp Ile Phe Arg Glu Thr Gly Asp
 225 230 235 240
 65 Asn Phe Ser Asp Val Ala Ile Gln Gly Gly Ile Met Gly Ile Glu Ile
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 260 265 270
 75 Tyr Ser Phe Arg Arg Leu Asp Asp Lys Thr Thr Asn Val Ser Leu Tyr
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 80 Pro Gly Tyr Asn Phe Arg Tyr Ala Lys Tyr Tyr Lys Glu Asn Asn Val
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Glu Lys Arg Thr Leu Ile Lys Val Phe Gly Ile Arg Phe Asp Ile Leu
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 30 Gly Ser Thr Leu Ser Tyr Phe Gly Leu Ala Ala Val Phe Ile Asp Phe
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 35 Leu Ile Asp Thr Tyr Ser Ser Asn Cys Cys Arg Ser His Ile Tyr Pro
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 40 Trp Cys Lys Cys Cys Gln Pro Cys Val Val Asn Glu Tyr Tyr Tyr Arg
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 45 Lys Lys Cys Glu Ser Ile Val Glu Pro Lys Pro Thr Leu Lys Tyr Val
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 50 Ser Phe Val Asp Glu Ser His Ile Arg Met Val Asn Gln Gln Leu Leu
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5 Cys Leu Pro Ser Gln Leu Pro Glu Ser His Arg Cys Leu Glu Glu Leu
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10 Cys Cys Arg Lys Lys Pro Gly Ala Cys Ile Thr Thr Ser Glu Leu Phe
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Arg Lys Leu Val Leu Ser Arg His Val Leu Gln Phe Leu Leu Leu Tyr
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15 Gln Glu Pro Leu Leu Ala Leu Asp Val Asp Ser Thr Asn Ser Arg Leu
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20 Arg His Cys Ala Tyr Arg Cys Tyr Ala Thr Trp Arg Phe Gly Ser Gln
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30 Pro Tyr
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 Phe His Val Ile Ile Phe Ser Tyr Val Cys Phe Ala Leu Val Ser Asp
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 10 Lys Leu Tyr Gln Arg Lys Glu Pro Val Ile Ser Ser Val His Thr Lys
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 Val Lys Gly Ile Ala Glu Val Lys Glu Glu Ile Val Glu Asn Gly Val
 65 70 75 80
 Lys Lys Leu Val His Ser Val Phe Asp Thr Ala Asp Tyr Thr Phe Pro
 85 90 95
 20 Leu Gln Gly Asn Ser Phe Phe Val Met Thr Asn Phe Leu Lys Thr Glu
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 10 Asn Phe Thr Val Leu Ile Lys Asn Ile Asp Phe Pro Gly His Asn
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 115 120 125
 His Lys Thr Gln Asn Pro Gln Cys Pro Ile Phe Arg Leu Gly Asp Ile
 130 135 140
 20 Phe Arg Glu Thr Gly Asp Asn Phe Ser Asp Val Ala Ile Gln Gly Gly
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 Ile Met Gly Ile Glu Ile Tyr Trp Asp Cys Asn Leu Asp Arg Trp Phe
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 25 His His Cys Arg Pro Lys Tyr Ser Phe Arg Arg Leu Asp Asp Lys Thr
 180 185 190
 30 Thr Asn Val Ser Leu Tyr Pro Gly Tyr Asn Phe Arg Tyr Ala Lys Tyr
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 Tyr Lys Glu Asn Asn Val Glu Lys Arg Thr Leu Ile Lys Val Phe Gly
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 35 Ile Arg Phe Asp Ile Leu Val Phe Gly Thr Gly Gly Lys Phe Asp Ile
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 40 Ile Gln Leu Val Val Tyr Ile Gly Ser Thr Leu Ser Tyr Phe Gly Leu
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 25 Cys Ser Ser Asp Arg Gly Cys Lys Lys Gly Trp Met Asp Pro Gln Ser
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 30 Thr Cys Glu Val Ser Ala Trp Cys Pro Ile Glu Ala Val Glu Glu Ala
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 40 Leu Pro Gly Leu Asn Ile Thr Cys Thr Phe His Lys Thr Gln Asn Pro
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 25 Leu Gln Gly Asn Ser Phe Phe Val Met Thr Asn Phe Leu Lys Thr Glu
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Claims

- 45
1. A peptide consisting of a sequence within the sequence shown in SEQ ID NO: 3, said peptide being useful as an immunogen to generate an antibody that is capable of selectively binding to a non ATP -binding P2X7 receptor but not to an ATP -binding P2X7 receptor, wherein the peptide includes the sequence shown in SEQ ID NO: 2.
 - 50 2. A peptide according to claim 1, wherein said peptide consists of the sequence shown in SEQ ID NO: 2.
 3. A peptide according to claim 1, wherein said peptide consists of the sequence shown in SEQ ID NO: 3.
 4. An isolated or purified antibody or fragment thereof capable of binding to a peptide consisting of the amino acid
55 sequence shown in SEQ ID NO: 2.
 5. An isolated or purified antibody or fragment thereof according to claim 4, wherein the antibody fragment is selected from the group consisting of a Fab, Fd, Fv, F(ab')₂ and scFv.

6. An immune complex including the sequence shown in SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 9 and an isolated or purified antibody or fragment thereof according to claim 4 or 5, wherein the antibody or fragment thereof binds to SEQ ID NO: 2 in any one of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 9.
- 5 7. A method for determining whether a cell, tissue or extra cellular body fluid includes a non ATP -binding P2X7 receptor, monomer or fragment thereof including:
- contacting a cell, tissue or extra cellular body fluid *in vitro* with an antibody or antigen-binding fragment thereof of claim 4 or 5 in conditions for forming an immune complex with a non ATP -binding P2X7 receptor, monomer or fragment thereof, and
 - detecting whether an immune complex has been formed,
- 10
- wherein detection of an immune complex determines that a cell, tissue or extra - cellular body fluid includes a non ATP -binding P2X7 receptor, monomer or fragment thereof.
- 15
8. A method for determining whether an extra -cellular body fluid contains an antibody against a non ATP -binding P2X7 receptor, monomer or fragment thereof including:
- contacting an extra -cellular body fluid *in vitro* with a peptide according to any one of claims 1 to 3 in conditions for forming an immune complex, and
 - detecting whether an immune complex has been formed between the peptide according to any one of claims 1 to 3 and an antibody against a non ATP - binding P2X7 receptor, monomer or fragment thereof,
- 20
- wherein detection of an immune complex determines that an extra -cellular body fluid contains an antibody against a non ATP -binding P2X7 receptor, monomer or fragment thereof.
- 25
9. A pharmaceutical composition including an antibody or antigen-binding fragment thereof according to claim 4 or 5, together with a pharmaceutically acceptable carrier, diluent or excipient.
- 30
10. A pharmaceutical composition including a peptide according to any one of claims 1 to 3 together with a pharmaceutically acceptable carrier, diluent or excipient.
11. Use of an antibody according to claim 4 or 5 in the manufacture of a medicament for the treatment of cancer.
- 35
12. An antibody according to claim 4 or 5 for use in the treatment of cancer.

Patentansprüche

- 40
1. Peptid, das aus einer Sequenz innerhalb der Sequenz besteht, die in SEQ ID NO: 3 gezeigt ist, wobei das genannte Peptid als Immunogen nützlich ist, um einen Antikörper zu erzeugen, der in der Lage ist, selektiv an einen nicht-ATP-bindenden P2X7-Rezeptor, jedoch nicht an einen ATP-bindenden P2X7-Rezeptor zu binden, wobei das Peptid die in SEQ ID NO: 2 gezeigte Sequenz umfasst.
- 45
2. Peptid nach Anspruch 1, wobei das genannte Peptid aus der Sequenz besteht, die in SEQ ID NO: 2 gezeigt ist.
3. Peptid nach Anspruch 1, wobei das genannte Peptid aus der Sequenz besteht, die in SEQ ID NO: 3 gezeigt ist.
4. Isolierter oder gereinigter Antikörper oder Fragment davon, der bzw. das in der Lage ist, an ein Peptid zu binden, dass aus der Aminosäuresequenz besteht, die in SEQ ID NO: 2 gezeigt ist.
- 50
5. Isolierter oder gereinigter Antikörper oder Fragment davon nach Anspruch 4, wobei das Antikörperfragment aus der Gruppe ausgewählt wird, die aus einem Fab, Fd, Fv, F(ab')₂ und scFv besteht.
- 55
6. Immunkomplex, der die Sequenz umfasst, die in SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 oder SEQ ID NO: 9 gezeigt ist, und isolierter oder gereinigter Antikörper oder Fragment davon nach Anspruch 4 oder 5, wobei der Antikörper oder das Fragment davon an SEQ ID NO: 2 in einer der SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 oder SEQ ID NO: 9 bindet.

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7. Verfahren zur Bestimmung, ob ein Zell-, Gewebe- oder extrazelluläres Körperfluid einen nicht-ATP-bindenden P2X7-Rezeptor, ein Monomer oder ein Fragment davon umfasst, wobei das Verfahren Folgendes umfasst:
- Inkontaktbringen eines Zell-, Gewebe- oder extrazellulären Körperfluids in vitro mit einem Antikörper oder Antigen-bindenden Fragment davon nach Anspruch 4 oder 5 unter Bedingungen zur Bildung eines Immunkomplexes mit einem nicht ATP-bindenden P2X7-Rezeptor, einem Monomer oder ein Fragment davon und
 - Nachweisen, ob ein Immunkomplex gebildet wurde, wobei der Nachweis eines Immunkomplexes bestimmt, dass ein Zell-, Gewebe- oder extrazelluläres Körperfluid einen nicht-ATP-bindenden P2X7-Rezeptor, ein Monomer oder ein Fragment davon umfasst.
8. Verfahren zur Bestimmung, ob ein extrazelluläres Körperfluid einen Antikörper gegen einen nicht-ATP-bindenden P2X7-Rezeptor, ein Monomer oder ein Fragment davon umfasst, wobei das Verfahren Folgendes umfasst:
- Inkontaktbringen eines extrazellulären Körperfluids in vitro mit einem Peptid nach einem der Ansprüche 1 bis 3 unter Bedingungen zur Bildung eines Immunkomplexes und
 - Nachweisen, ob ein Immunkomplex zwischen dem Peptid nach einem der Ansprüche 1 bis 3 und ein Antikörper gegen einen nicht-ATP-bindenden P2X7-Rezeptor, ein Monomer oder ein Fragment davon gebildet wurde,
- wobei der Nachweis eines Immunkomplexes bestimmt, dass ein extrazelluläres Körperfluid einen Antikörper gegen einen nicht-ATP-bindenden P2X7-Rezeptor, ein Monomer oder ein Fragment davon umfasst.
9. Pharmazeutische Zusammensetzung, die einen Antikörper oder ein Antigen-bindendes Fragment davon nach Anspruch 4 oder 5 sowie einen pharmazeutisch verträglichen Träger, ein pharmazeutisch verträgliches Verdünnungsmittel oder einen pharmazeutisch verträglichen Hilfsstoff umfasst.
10. Pharmazeutische Zusammensetzung, die ein Peptid nach einem der Ansprüche 1 bis 3 sowie einen pharmazeutisch verträglichen Träger, ein pharmazeutisch verträgliches Verdünnungsmittel oder einen pharmazeutisch verträglichen Hilfsstoff umfasst.
11. Verwendung eines Antikörpers nach Anspruch 4 oder 5 zur Herstellung eines Medikaments zur Behandlung von Krebs.
12. Antikörper nach Anspruch 4 oder 5 zur Verwendung bei der Behandlung von Krebs.

Revendications

1. Peptide constitué d'une séquence faisant partie de la séquence représentée dans la SEQ. ID n° 3, ledit peptide pouvant être utilisé comme un immunogène pour produire un anticorps qui est capable de se lier sélectivement à un récepteur P2X7 qui ne se lie pas à l'ATP, mais pas à un récepteur P2X7 qui se lie à l'ATP, le peptide comprenant la séquence représentée dans la SEQ. ID n° 2.
2. Peptide selon la revendication 1, ledit peptide étant constitué de la séquence représentée dans la SEQ. ID n° 2.
3. Peptide selon la revendication 1, ledit peptide étant constitué de la séquence représentée dans la SEQ. ID n° 3.
4. Anticorps isolé ou purifié ou fragment de celui-ci capable de se lier à un peptide constitué de la séquence d'acides aminés représentée dans la SEQ. ID n° 2.
5. Anticorps isolé ou purifié ou fragment de celui-ci selon la revendication 4, le fragment d'anticorps étant sélectionné dans le groupe constitué d'un fragment Fab, Fd, Fv, F(ab')₂ et scFv.
6. Complexe immun comprenant la séquence représentée dans la SEQ. ID n° 4, la SEQ. ID n° 5, la SEQ. ID n° 6 ou la SEQ. ID n° 9 et un anticorps isolé ou purifié ou un fragment de celui-ci selon la revendication 4 ou 5, l'anticorps ou le fragment de celui-ci se liant à la SEQ. ID n° 2 dans l'une quelconque de la SEQ. ID n° 4, la SEQ. ID n° 5, la SEQ. ID n° 6 ou la SEQ. ID n° 9.
7. Procédé permettant de déterminer si une cellule, un tissu ou un liquide corporel extracellulaire contient ou non un

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récepteur P2X7 qui ne se lie pas à l'ATP, un monomère ou un fragment de celui-ci, comprenant :

- la mise en contact d'une cellule, d'un tissu ou d'un liquide corporel extracellulaire *in vitro* avec un anticorps ou un fragment de celui-ci se liant à un antigène selon la revendication 4 ou 5, dans des conditions permettant de former un complexe immun avec un récepteur P2X7 qui ne se lie pas à l'ATP, un monomère ou un fragment de celui-ci, et
- une détection pour déterminer si un complexe immun a été formé ou non,

dans lequel la détection d'un complexe immun détermine qu'une cellule, un tissu ou un liquide corporel extracellulaire contient un récepteur P2X7 qui ne se lie pas à l'ATP, un monomère ou un fragment de celui-ci.

8. Procédé permettant de déterminer si un liquide corporel extracellulaire contient ou non un anticorps dirigé contre un récepteur P2X7 qui ne se lie pas à l'ATP, un monomère ou un fragment de celui-ci, comprenant :

- la mise en contact d'un liquide corporel extracellulaire *in vitro* avec un peptide selon l'une quelconque des revendications 1 à 3, dans des conditions permettant de former un complexe immun, et
- une détection pour déterminer si un complexe immun a été formé ou non entre le peptide selon l'une quelconque des revendications 1 à 3 et un anticorps dirigé contre un récepteur P2X7 qui ne se lie pas à l'ATP, un monomère ou un fragment de celui-ci,

dans lequel la détection d'un complexe immun détermine qu'un liquide corporel extracellulaire contient un anticorps dirigé contre un récepteur P2X7 qui ne se lie pas à l'ATP, un monomère ou un fragment de celui-ci.

9. Composition pharmaceutique contenant un anticorps ou un fragment de celui-ci se liant à un antigène selon la revendication 4 ou 5, conjointement avec un support, un diluant ou un excipient pharmaceutiquement acceptable.

10. Composition pharmaceutique contenant un peptide selon l'une quelconque des revendications 1 à 3 conjointement avec un support, un diluant ou un excipient pharmaceutiquement acceptable.

11. Utilisation d'un anticorps selon la revendication 4 ou 5 dans la fabrication d'un médicament pour le traitement du cancer.

12. Anticorps selon la revendication 4 ou 5, destiné à une utilisation dans le traitement du cancer.

Figure 1: SEQ ID NO:1

1 MPACCSQSDV FQYETNKVTR IQSMNYGTIK WFFHVIIFS Y VCFALVSDKL YQRKEPVISS
61 VHTKVKGIAE VKEEIVENG V KKL VHSVFD T ADYTFPLQGN SFFVMTNFLK TEGQEQLRCP
121 EYPTRRTLCS SDRGCKKGWM DPQSKGIQTG RCVVHEGNQK TCEVSAWCPI EAVEEAPRPA
181 LLNSAENFTV LIKNNIDFPG HNYTTRNILP GLNITCTFHK TQNPQCPIFR LGDIFRETGD
241 NFSDVAIQGG IMGIEIYWDC NLD RWFHHC R PKYSFRRLDD KTTNVS LYPG YNFRYAKYYK
301 ENNVEKRTLI KVEGIRFDIL VFGTGGKFDI IQLVVYIGST LSYFGLAAVF IDFLIDTYSS
361 NCCRSHIYPW CKCCQPCVVN EYYYRKKCES IVEPKPTLKY VSFVDESHIR MVNQQLGRS
421 LQDVKGQEV P RPAMDFTDLS RLPLALHDT P PIPGQPEEQ LLRKEATPRS RDSPVWCQCG
481 SCLPSQLPES HRCLEELCCR K KPGACITTS ELFRKLVLSR HVLQFLLLYQ EPLLALDVDS
541 TNSRLRHCA Y RCYATWRFGS QDMADFAILP SCCRWRIRKE FPKSEGQYSG FKSPY

Figure 2: SEQ ID NO:2

KYYKENNVEKRTLKVF

Figure 3: SEQ ID NO:3

KTTNVSLYPGYNFRYAKYYKENNVEKRTLKVFGRFDILVFGTGGKFD

Figure 4: SEQ ID NO:4

MTPGDHSWGN SFFVMTNFLK TEGQEQLCP

121 EYPTRRTLCS SDRGCKKGWM DPQSKGIQTG RCVVHEGNQK TCEVSAWCPI EAVEEAPRPA

181 LLNSAENFTV LIKNNIDFPG HNYTTRNILP GLNITCTFHK TQNPQCPIFR LGDIFRETGD

241 NFSDVAIQGG IMGIEIYWDC NLDRAWFHHCR PKYSFRRLDD KTTNVSLYPG YNFRYAKYYK

301 ENNVEKRTLI KVFGRFDIL VFGTGGKFDI IQLVVYIGST LSYFGLAAVF IDFLIDTYSS

361 NCCRSHIYPW CKCCQPCVVN EYYRKKCES IVEPKPTLKY VSFVDESHIR MVNQQLGRS

421 LQDVKGQEVV RPAMDFTDLS RLPLALHOTP PIPGQPEEQ LLRKEATPRS RDSPVWCQCG

481 SCLPSQLPES HRCLEELCCR KKPACITTS ELFRKLVLSR HVLQFLLLYQ EPLLALDVDS

541 TNSRLRHCAV RCYATWRFVS QDMADFAILP SCCRWIRKE FPKSEGQYSG FKSPY

Figure 5: SEQ ID NO:5

MDGPAEQRPA

181 LLNSAENFTV LIKNNIDFPG HNYTTRNILP GLNITCTFHK TQNPQCPIFR LGDIFRETGD

241 NFSDVAIQGG IMGIEIYWDC NLDRAWFHCR PKYSFRRLDD KTTNVSLYPG YNFRYAKYYK

301 ENNVEKRTLI KVFGRFDIL VFGTGGKFDI IQLVVYIGST LSYFGLAAVF IDFLIDTYSS

361 NCCRSHIYPW CKCCQPCVVN EYYRKKCES IVEPKPTLKY VSFVDESHIR MVNQQLGRS

421 LQDVKGQEVV RPAMDFTDLS RLPLALHOTP PIPGQPEEIQ LLRKEATPRS RDSPVWCQCG

481 SCLPSQLPES HRCLEELCCR KKPACITTS ELFRKLVLSR HVLQFLLLYQ EPLLALDVDS

541 TNSRLRHCAV RYATWRFGS QDMADFAILP SCCRWIRKE FPKSEGQYSG FKSPY

Figure 6: SEQ ID NO:6

1 MPACCSCSDV FQYETNKVTR IQSMNYGTIK WFFHVIIFS Y VCFALVSDKL YQRKEPVISS

61 VHTKVKGIAE VKEEIVENG V KKLVSFVFD T ADYTFPLQGN SFFVMTNFLK TEGQEQLCP

121 EYPTRRTLCS SDRGCKKGWM DPQSKGIQTG RCVVHEGNQK TCEVSAWCPI EAVEEAPRPA

181 LLNSAENFTV LIKNNIDFPG HNYTTRNILP GLNITCTFHK TQNPQCPIFR LGDIFRETGD

241 NFSDVAIQGG IMGIEIYWDC NLDRAWFHCR PKYSFRRLDD KTTNVSLYPG YNFRYAKYYK

301 ENNVEKRTLI KVFGRFDIL VFGTGGKFDI IQLVVYIGST LSYFGLVRDS EGSD

Figure 7: SEQ ID NO:7

M WQFRYAKYYK

301 ENNVEKRTLI KVFGRFDIL VFGTGGKFDI IQLVVYIGST LSYFGLAAVF IDFLIDTYSS

361 NCCRSHIYPW CKCCQPCVVN EYYRKKCES IVEPKPTLKY VSFVDESHIR MVNQQLGRS

421 LQDVKGQEVV RPAMDFTDLS RLPLALHDTF PIPGQPEEQ LLRKEATPRS RDSPVWCQCG

481 SCLPSQLPES HRCLEELCCR KKPACITTS ELFRKLVLSR HVLQFLLLYQ EPLLALDVDS

541 TNSRLRHAY RYATWRFGS QDMADFAILP SCCRWIRKE FPKSEGQYSG FKSPY

Figure 8: SEQ ID NO:8

1 MPACCSCSDV FQYETNKVTR IQSMNYGTIK WFFHVIIFS Y VCFALVSDKL YQRKEPVISS

61 VHTKVKGIAE VKEEIVENG V KKLVSFVDT ADYTFPLQGN SFFVMTNFK TEGQEQLCP

121 EYPTRRTLCS SDRGCKKGWM DPQSKGIQTG RCVVHEGNQK TCEVSAWCPI EAVEEAPRPA

181 LLNSAENFTV LIKNNIDFPG HNYTT

241 YAKYYK

301 ENNVEKRTLI KVFGRFDIL VFGTGGKFDI IQLVVYIGST LSYFGLVRDS LFHALGKWF

361 EGSD

Figure 9: SEQ ID NO:9

61 MTPGDHSWGN SFFVMTNFLK TEGQEQLCP
121 EYPTRRTLCS SDRGCKKGWM DPQSKGIQTG RCVVHEGNQK TCEVSAWCPI EAVEEAPRPA
181 LLNSAENFTV LIKNNIDFPG HNYTTRNILP GLNITCTFHK TQNPQCPIFR LGDIFRETGD
241 NFSDVAIQGG IMGIEIYWDC NLDRWFHCR PKYSFRLDD KTTNVSLYPG YNFRYAKYYK
301 ENNVEKRTLI KVFIRFDIL VFGTGGKFDI IQLVVIYIGST LSYFGLVRDS LFHALGKWFG
361 EGSD

Figure 10: SEQ ID NO:10

1 MPACCSCSDV FQYETNKVTR IQSMNYGTIK WFFHVIIFSY VCFALVSDKL YQRKEPVISS
61 VHTKVKGIAE VKEEIVENG V KKLVSFVDT ADYTFPLQGN SFFVMTNFLK TEGQEQLCP
121 EYPTRRTLCS SDRGCKKGWM DPQSKGIQTG RCVVHEGNQK TCEVSAWCPI EAVEEAPRPA
181 LLNSAENFTV LIKNNIDFPG HNYTTRNILP GLNITCTFHK TQNPQCPIFR LGDIFRETGD
241 NFSDVAIQIR QVLQKQC

Figure 11: SEQ ID NO:11

1 MPACCSCSDV FQYETNKVTR IQSMNYGTIK WFFHVIIFS Y VCFALVSDKL YQRKEPVISS
61 VHTKVKGIAE VKEEIVENG V KKL VHSVFD T ADYTFPLQGN SFFVMTNFLK TEGQEQLCP
121 EEF RPEGV

Figure 12: SEQ ID NO:12

GHNYTTRN ILPGLNITC

REFERENCES CITED IN THE DESCRIPTION

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